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Applicant:
(Name and address)

NOVO NORDISK A/S
Novo Allé
DK-2880 Bagsværd
Denmark

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Helle Schackinger Olesen

Helle Schackinger Olesen

Patent- og Varemærkestyrelsen

PREVENTION OF HYPERINSULINEMIA IN SUBJECTS UNDERGOING GROWTH HORMONE (GH) TREATMENT

BACKGROUND OF THE INVENTION

Obesity is known to be associated with serious risk factors, and there is currently intense interest in identifying new principles for treatment of this condition. These efforts have hitherto resulted in identification of a substantial number of potential central and peripheral targets for treatment (1). It has also been shown that growth hormone (GH), more specifically human growth hormone (hGH) in human beings, acts as a potent regulator of body fat storage, and thus promotes breakdown of adipose tissue in obese humans while preserving lean tissues (2-7). Since a large proportion of glucose disposal and energy expenditure is thought to take place in lean tissues, preservation of such tissues combined with a selective loss of adiposity appears to be a highly desirable objective.

Although it is known that the protein anabolic aspect of the GH - insulin like growth factor-1 axis is influenced by diet composition (8-10), there does not appear to have been any focus on the question of whether this is also true for GH-stimulated loss of adipose tissue. The effect of GH during restriction of energy intake has also been unclear. There have been some reports indicating no additional effects of GH administration compared to the effect of energy restriction alone (11-13), whilst other reports have indicated the such effects (4,7).

A recently reported study by the present inventors and co-workers (18) indicated GH-stimulated breakdown of adipose tissue in genetically intact old rats that had become obese while receiving a high-fat diet. However, despite normalisation of body fat stores following GH injection, basal insulin levels were significantly elevated.

It has been suggested that an excessive hyperinsulinemic response to GH injections would decrease the net effect of GH on adipose tissue (11,14). This would not be surprising since GH and insulin have been suggested to have opposing effects in adipose tissue (15). Moreover, sustained hyperinsulinemia would also increase the risk of development of overt Type 2 diabetes in susceptible obese individuals that already are at risk (16,17).

There is thus a clear need to identify factors determining the insulin response to administration of GH, both from a mechanistic and a safety point of view. The present inventors have now obtained clear indications that the macronutrient composition of the diet received by obese individuals constitutes one such factor, and that total energy intake constitutes another.

SUMMARY OF THE INVENTION

The present inventors have found that the insulin response in a subject to administration of GH can be modulated, for example, by varying diet composition and caloric intake, and/or by administering a drug which brings about a reduction in blood lipid levels, and that this influences adipose tissue loss and serum leptin levels. A broad aspect of the invention thus relates to a method for substantially preventing hyperinsulinemia in an animal or human subject undergoing treatment with growth hormone (GH), the method comprising subjecting the subject, during the growth hormone treatment period, to one or more measures (such a diet regimen and/or a drug treatment) which cause a reduction in blood lipid levels. Further aspects of the invention include, *inter alia*:

(i) a method for achieving breakdown of adipose tissue in an animal or human subject substantially without induction of hyperinsulinemia in the subject, the method comprising administering a growth hormone (GH) to the subject whilst subjecting the subject to one or more measures which cause a reduction in blood lipid levels;

(ii) a method for reducing blood lipid levels in an animal or human subject substantially without induction of hyperinsulinemia in the subject, the method comprising administering a growth hormone (GH) to the subject whilst inhibiting lipolysis in the subject; and

(iii) a method for reducing blood lipid levels in an animal or human subject substantially without induction of hyperinsulinemia in the subject, the method comprising administering a growth hormone (GH) to the subject whilst stimulating lipid clearance from circulation.

Other aspects of the invention include medical kits suitable for use in methods according to the invention.

Further detailed aspects of the invention are described below.

LIST OF FIGURES

Fig. 1. Intakes of food and metabolizable energy, and body weight development during the "fattening" period before GH dosing in old rats fed a high-fat (HF) diet (□-□, n=58) or a low-fat (LF) diet (○-○, n=23). Data represent means ± SE.

Fig. 2. Adipose tissue weight in relation to body weight and plasma concentrations of leptin and insulin, in old rats injected with growth hormone (GH) or saline (Sal). LF/LF signifies that animals were fed a low-fat (LF) diet both in the "fattening" period before GH dosing and during a 3-week GH-dosing period. By analogy, HF/HF signifies that a high-fat (HF) diet was provided in both periods, whereas HF/LF signifies that rats were shifted from the HF diet to the LF diet as GH dosing began. The suffixes/subscripts "re" and "pf" denote diet restriction and pair-feeding, respectively (for details, see Table 2). Data represent means ± SE (n=11-12).

Fig. 3. Plasma insulin-like growth factor-1 (IGF-1) levels in old rats injected with growth hormone (GH) or with saline (Sal). LF/LF signifies that animals were fed a low-fat (LF) diet both in the "fattening" period before GH dosing and during a 3-week GH-dosing period. By analogy, HF/HF signifies that a high-fat (HF) diet was provided in both periods, whereas HF/LF signifies that rats were shifted from the HF diet to the LF diet as GH dosing began. The suffixes/subscripts "re" and "pf" denote diet restriction and pair-feeding, respectively (for details, see Table 2). Data represent means ± SE (n=11-12).

Fig. 4. Plasma concentrations of glucose in old rats injected with growth hormone (GH) or with saline (Sal). LF/LF signifies that animals were fed a low-fat (LF) diet both in the "fattening" period before GH dosing and during a 3-week GH-dosing period. By analogy, HF/HF signifies that a high-fat (HF) diet was provided in both periods, whereas HF/LF signifies that rats were shifted from the HF diet to the LF diet as GH dosing began. The suffixes/subscripts "re" and "pf" denote diet restriction and pair-feeding, respectively (for details, see Table 2). Data represent means ± SE (n=11-12).

MATERIALS AND METHODS

Animals and test substances

Female Wistar rats weighing about 260 g were purchased 1 month before the start of experiments from Møllegård Breeding and Research Centre (Lille Skensved, Denmark). On arrival, rats were placed in conventional rat cages housing 2-3 animals. They were weighed weekly, and had free access to drinking water and a standard rat feed. All diets, including the experimental diets (see Table 1, below), were purchased from a local feed supplier (Brogaarden, Gentofte, Denmark). The experimental protocol was approved by the Danish national ethical committee for animal experiments (Dyreforsøgstilsynet, Copenhagen, Denmark). The growth hormone (GH) used in the present study was recombinantly produced human GH (hGH) from Novo Nordisk A/S (Bagsværd, Denmark).

Experimental procedures

At an age of 12 months, rats were randomly assigned to receive either a high-fat (HF) diet [number of rats (n) =58] or a low-fat (LF) diet (n=23) (Table 1).

Table 1. Composition of high-fat (HF) and low-fat (LF) diets with theoretical energy content (percent) in parentheses.

	Diets	
Ingredients (g/kg)	**HF**	**LF**
Maize meal	493	818
Wheat bran	27	27
Casein	148	110
Animal fat	300	13
Vitamins and minerals	32	32
Chemical composition (g/kg)		
Crude protein (energy %)	170 (18%)	170 (25%)
Crude fat (energy %)	320 (55%)	50 (12%)
Carbohydrates, total	344 (27%)	565 (63%)
Crude fibre	17	26
Disaccharides	11	18
Polysaccharides	316	521
Metabolizable energy (Mcal/kg)	4.8	3.2
Fatty acids		
Total (% of crude fat)	94	92
Saturated (% of total)	49	25
Mono-unsaturated (% of total)	39	30
Poly-unsaturated (% of total)	12	45

The diet in question was continued for 14 weeks, during which body weight development was recorded (Fig. 1). During this time, rats were assigned to 8 groups for dosing of GH thereafter, as indicated in Table 2.

Table 2. Allocation of rats to high-fat (HF) and low-fat (LF) diets during a 14-week period before dosing and during a 3-week period of dosing with either saline or growth hormone (GH). GH was administered in a total dose of 4 mg/kg/day, divided into two injections.

Diet before dosing	Diet during dosing	Dosing	n	Denomination
Low Fat	Low Fat	Saline	11	LF/LF-Sal
		GH	12	LF/LF-GH
High Fat	High Fat	Saline	12	HF/HF-Sal
		GH	11	HF/HF-GH
	Energy restricted*	GH	12	HF/HF-GHre
	Low Fat	Saline	11	HF/LF-Sal
		GH	12	HF/LF-GH
	Pair fed*	Saline	12	HF/LF-Salpf

*Age- and weight-matched rats were fed the same amount of metabolizable energy consumed by HF/LF-GH-group. In the HF/HF-GHre group, this was achieved by restricting food intake (energy-restricted), whereas rats in the HF/LF-Salpf group were pair-fed. Both these groups were run behind the others.

Injection of GH continued for 3 weeks, after which the rats were sacrificed by decapitation. After bleeding, serum and plasma were prepared and frozen at -80 °C.

The large fat pad embedding the kidneys (denoted peri-renal fat), and fat pads around the uterus, ovaries and intestines (denoted body fat or adipose tissue) were quickly dissected. After dissection the adipose tissue was weighed and frozen at –80 °C. One thigh muscle, the *quadriceps femoris*, was also dissected and weighed.

Chemical analyses

The content of fat in muscle tissue was analysed by Bioteknologisk Institut (Kolding, Denmark) using gas chromatography and employing standardized methods. Plasma concentrations of metabolites were analysed with a Synchron™ CX5 auto-analyzer system (Beckman Instruments, Fullerton, USA).

Hormone assays

Total plasma IGF-I was measured after acid-ethanol extraction as previously described (19); the intra-assay and inter-assay coefficients of variation were 6% and 13%, respectively. Plasma insulin was determined using an assay described previously (20); the intra- and inter-assay coefficients of variation for this assay were 5.4% and 8.4% respectively. Plasma leptin was determined using a commercial kit from Linco research, Inc. (St Charles, USA); the intra- and inter-assay coefficients of variation were 4.6% and 5.7%, respectively.

Statistical analyses

All experimental data were entered into the 6.11 version of the SAS statistical software program, whereby descriptive statistics were calculated using the univariate procedure (SAS Inc, Cary, USA). Before further analysis, data were checked for normal distribution. In some cases where deviations were found, data were logarithmically transformed to achieve such a distribution. Potential differences between treatment groups were tested with a one-way analysis of variance (GLM procedure of SAS) followed by Duncan's multiple-range test. In most cases these tests were performed with an α-value of 0.05, but where applicable an α-value of 0.01 was used. Data are presented as means ± standard error (SE).

RESULTS

Body weight gain and feed consumption in the period before GH dosing

At the start of the 14-week "fattening" period, rats in the HF group and the LF group had body weights of 279±3 and 280±6 g, respectively. Rats in the HF group gained 111±5 g in body weight during the 14 week period. Rats receiving the LF diet gained 63±5 g, which was significantly ($p<0.01$) lower. This resulted in significant ($p<0.01$) differences in live weights, viz. 390±6.4 (HF diet group) *vs.* 343±8.6 g (LF diet group).

As can be seen from Fig. 1, rats given the HF diet had a comparatively high intake of food, and consequently a comparatively high metabolizable energy intake in the first phase of the "fattening" period. This was followed by a clear decline, so that in the last phase, intakes of metabolizable energy in the two groups were similar.

Food consumption during GH dosing

As was the case during the "fattening" period, rats fed the LF diet during GH dosing consumed significantly ($p<0.05$) more food than did rats fed the HF diet (see Table 3, below), but due to the lower energy content of the LF diet the intakes of metabolizable energy were not significantly different between the two groups.

There was a general decrease in food intake in the first phase after commencement of GH injections (data not shown), thus confirming our previous observations (18). Measured over the three-week dosing period, this effect remained statistically significant for rats fed the LF diet (Table 3). GH-treated rats which were switched from the HF diet to the LF diet successively increased their consumption between the second and third week of treatment, ending up with about the same total amount of food ingested as the corresponding saline-control rats. During this phase the pair-fed group could not follow the GH-treated group, and small food refusals were occasionally observed, resulting in a slightly lower total consumption of food (Table 3).

Table 3 Effects of growth hormone (GH) dosing on: consumption of high-fat (HF) and low-fat (LF) diets, body weights, adipose tissue, skeletal muscle weight and muscle content of fat. Food intake was registered per cage unit containing 2-3 animals (n=6), and fat in muscle was analysed on a randomly selected number (n=5) of animals from each group. In other cases, means ± SE are based on 11-12 observations.

			Intakes						Body weight						Tissue weights and composition					
Diet before dosing	Diet during dosing	Dosing	Food (g/kg/d)		Metabolizable energy (Cal/kg/d)		Protein (g/kg/d)		Start weight (g)		Final weight (g)		Change in weight (g)		Adipose tissue (g)		Skeletal Muscle (g)		Fat in Muscle (%)	
Low-fat	Low-fat	Saline	46^{a}	±2.0	149^{ab}	±6.5	7.8^{a}	±0.34	345^{bc}	±13	355^{c}	±11	9.7^{d}	±3.6	41^{bc}	±3.8	2.3^{b}	±0.10	4.4^{a}	±1.6
		GH	40^{b}	±1.9	129^{bc}	±6.1	6.8^{b}	±0.32	341^{c}	±12	419^{abc}	±11	78^{a}	±3.7	30^{de}	±2.3	3.0^{a}	±0.07	1.7^{c}	±0.2
High-fat	High-fat	Saline	33^{cd}	±1.7	158^{a}	±8.0	5.6^{cd}	±0.28	391^{ab}	±17	400^{bcd}	±16	9.0^{d}	±3.7	60^{a}	±4.7	2.3^{b}	±0.08	3.1^{abc}	±0.9
		GH	29^{d}	±1.5	142^{ab}	±7.2	5.0^{d}	±0.25	390^{ab}	±15	452^{a}	±13	62^{b}	±4.9	38^{cd}	±2.7	2.8^{a}	±0.11	1.9^{bc}	±0.4
	Energy restricted*	GH	22^{*}	±0.0	106^{*}	±0.12	3.7^{*}	±0.00	389^{ab}	±9	429^{ab}	±9	40^{c}	±7.6	26^{e}	±1.6	3.0^{a}	±0.16	1.8^{bc}	±0.1
High-fat	Low-fat	Saline	37^{bc}	±2.4	119^{cd}	±7.9	6.2^{bc}	±0.42	388^{ab}	±17	381^{cde}	±16	-7^{e}	±3.6	50^{ab}	±4.5	2.2^{b}	±0.09	3.8^{ab}	±0.6
		GH	33^{cd}	±2.8	106^{d}	±9.0	5.6^{cd}	±0.47	389^{ab}	±16	431^{ab}	±10	41^{c}	±8.2	31^{cde}	±2.7	2.9^{a}	±0.07	1.6^{c}	±0.3
	Pair fed*	Saline	30^{*}	±2.1	97^{*}	±6.7	5.1^{*}	±0.35	393^{a}	±17	371^{de}	±15	-22^{e}	±5.4	49^{b}	±3.8	2.2^{b}	±0.05	2.8^{abc}	±0.4

* Groups that did not have free access to food were excluded from statistical analyses of food intake variables.

^{a,b,c,d} Differences between groups were tested with a one-way analysis of variance followed by Duncan's multiple range-test. Values within columns not sharing a common letter superscript differ significantly ($p<0.05$).

Body weight during dosing

Irrespective of diet, three weeks of GH treatment generally increased body weight (Table 3). A change of diet from HF to LF, combined with saline alone, produced a significant ($p<0.05$) reduction of live weight. Rats with the same dietary record which were pair fed with GH-treated counterparts also lost weight (Table 3).

Effects of diet and GH on body composition

GH treatment significantly ($p<0.05$) decreased the weight of adipose tissue excised from rats fed HF or LF diet, or rats that were switched from HF to LF, both in absolute numbers (Table 3) and in relation to their body weight (Fig. 2). Pair-feeding alone did not significantly affect the weight of adipose tissue. In parallel with the reductions of adipose tissue weight seen in animals injected with GH, fresh muscle (*Quadriceps femoris*) weights generally increased significantly ($p<0.05$) (Table 3). When expressed in relation to body weight, this effect was not statistically significant in rats that were on the HF diet throughout the entire study. The fat content of muscle tissue was generally decreased in all groups injected with GH, although the decrease was not statistically significant in all instances (Table 3).

Effects of GH on plasma variables

Injection with GH produced a significant ($p<0.05$) increase of plasma IGF-1 concentrations irrespective of dietary regimen (Fig. 3). However, the increase was significantly ($p<0.05$) lower in animals with restricted access to the HF diet. Exceptionally low insulin and leptin levels were also observed in this group (Fig. 2), and glucose levels in this group were concomitantly significantly decreased in comparison to any other group (Fig. 4). In contrast, GH administration to animals with free access to the HF diet produced a marked hyperinsulinemia, and no fall in leptin levels was seen; glucose levels in these animals were likewise not significantly changed compared with levels in saline-control animals.

DISCUSSION OF RESULTS

At the conclusion of the experiments described above, rats that had received the HF diet throughout contained about 30 % more adipose tissue than rats fed the LF diet. This difference is likely founded in the phase when rats first were introduced to a HF diet, since in that phase their daily intake of metabolizable energy was clearly elevated for several weeks. After about 6 weeks, their caloric intake had fallen to the same level as that of rats fed the LF diet, indicating that although this adaptation is not as fast as has been observed in young animals (21), the old rats employed in the present work still retain some ability to regulate their caloric intake.

Leptin, produced by adipose tissue, is a functional component in energy homeostasis and has both central (22) and peripheral (23) effects. It is not therefore not surprising that dietary-induced obesity usually is associated with significant increases in plasma leptin levels (24,25). This was confirmed in the work described herein, where rats fed the HF diet exhibited significantly elevated leptin levels. It is thus most likely that leptin participates in the down-regulation of food intake that was observed in animals fed the HF diet. However, the present work and other studies (26) indicate that the leptin system does not provide complete protection against development of obesity. One reason for this could be that a certain degree of adiposity is needed to produce leptin levels that exceed the threshold for activation of central feeding centres. Another reason might be that the leptin-mediated stimulation of lipid oxidation in skeletal muscle decreases after prolonged exposure to high levels of the hormone (27), and that this might increase the efficiency by which adipose stores are built up from a given amount of substrate.

Injections of GH produced an increase in skeletal muscle weight, loss of adipose tissue and a transient decrease in food intake (data not shown) in those groups of animals that had free access to food. These effects were observed irrespective of whether rats were maintained on their habitual diets or were shifted from the HF to the LF diet. Shift of diet without GH injection, but with essentially the same caloric intake (pair-fed), produced a fall in body weight, but only marginal effects on adipose tissue weight. Such a "defence" of body composition has been reported previously (28,29). In view of this, it can be concluded that treatment with GH produces a significant and specific loss of adipose tissue, thus verifying our previous findings (18). It also appears that promotion of fat loss is a consistent effect of GH under a variety of dietary conditions, but that modulation of this effect by the amount of diet eaten occurs. Thus, if the HF diet is fed in restricted amounts, the fat loss after GH injections is greater than if there is free access to the same diet (and thereby is greater consumption of metabolizable energy). This observation shows that caloric intake either directly or indirectly modulates the effect of GH on fat loss.

In obese humans on caloric (energy) restriction, GH treatment has on some occasions been found to accelerate loss of body fat (4,7) compared with energy restriction alone, whereas others studies have failed to show such effects (12,13). It has been suggested that it is the magnitude of insulin response to GH injection that determines whether GH is able to accelerate loss of fat or not (11, 15). However, no experiments designed to prove this have to our knowledge been published, although it is known that GH accelerates loss of fat in energy-restricted Type 2 diabetics. The results presented herein indicate significant losses of adipose tissue in the face of very different insulin responses, and this may possibly originate in the use of a relatively high dose of GH in the present work, thereby overriding a possible counteractive effect of insulin. However, as described further below, the present study also provides support for GH-induced loss of adipose tissue stimulated by low plasma levels of insulin.

Thus, astonishingly low plasma insulin levels were found in blood from animals which had been fed restricted amounts of the HF diet during GH treatment, whereas hyperinsulinemia was especially marked in the case of animals which had had free access to the same diet. As mentioned before, the degree of adipose tissue breakdown was significantly different between these groups. The present inventors thus believe that they have identified a dietary situation whereby the well-known (30) hyperinsulinemic response to GH administration may be avoided, thus presenting the possibility of increasing both the efficacy and safety aspects of GH treatment of obese humans. There is little doubt that prolonged and uncontrolled hyperinsulinemia represents a real hazard to the patient (16) and must be taken seriously. With reference to the results described above concerning leptin levels in GH-treated subjects, it may be mentioned that there have been several reports of falls in plasma leptin levels and expression after GH treatment (31,32), whilst other reports have pointed to a more complex response pattern (33,34). In the work described herein, the GH-mediated loss of body fat is associated with variable responses in plasma leptin levels, depending on the type of diet and whether the animals had free (unrestricted) access to diet or were fed restricted amounts. In GH-treated animals that had unrestricted access to the HF diet throughout the study, plasma leptin levels did not fall despite a significant reduction of body fat stores. This is very surprising, since GH has been shown to inhibit leptin gene expression in other rodent models of obesity (31). Once again, however, restricted intake of the HF diet during GH treatment completely changed the response, and a remarkable fall in plasma leptin levels was observed. Although the loss of adipose tissue in this group was accelerated, it may be questioned whether this alone can explain the striking drop in plasma leptin concentration. Instead, it appears that plasma leptin levels are related not only to adiposity, but also to diet macronutrient composition (35,36) and - as observed in the present work - to daily caloric intake. There is accumulating evidence that insulin may be involved in this type of regulation of leptin expression (37,38), and the present

inventors believe that the results described herein support the conclusion that hyperinsulinemia prevents a fall in leptin levels, despite massive loss of adipose tissue.

To summarize, the results reported herein demonstrate not only that GH mediates breakdown of adipose tissue under a variety of dietary conditions, but – very importantly and surprisingly - that induction of hyperinsulinemia can be prevented if GH treatment is combined with restricted feeding of a diet which is relatively low in carbohydrates and rich in fat, and that same treatment regimen also promotes a fall in plasma leptin levels. The present invention is thus arrived at on the basis hereof.

DETAILED DESCRIPTION OF THE INVENTION

Certain aspects of the invention have already been outlined briefly above. Common for methods according to the invention as described below is the resulting lack of induction of hyperinsulinemia in the subject, thereby minimizing the risk of development of diabetes as a consequence of excessive overloading of insulin-producing beta-cells.

One aspect of the present invention relates to a method for substantially preventing hyperinsulinemia in an animal or human subject undergoing treatment with growth hormone (GH), the method comprising subjecting the subject, during the growth hormone treatment period, to one or measures which cause a reduction in blood lipid levels. The method in question is believed to be of general applicability, irrespective of the underlying rationale for treatment of the subject with growth hormone.Thus, for example, the method is anticipated to be of value in acknowledged/established GH treatments of immature humans (children or adolescents), such as for the purpose of stimulating growth to counteract development of short stature or dwarfism, as well as of mature (adult) humans.

A closely related, and particularly valuable, further aspect of the invention relates to a method for achieving breakdown of adipose tissue in an animal or human subject – particularly an obese human subject - substantially without induction of hyperinsulinemia in the subject, the method comprising administering a growth hormone (GH) to the subject whilst subjecting the subject to one or more measures which cause a reduction in blood lipid levels.

Measures of the type referred to in relation to methods of the invention are suitably diet regimens or drug treatments. As demonstrated in the present application, a diet regimen which has been found to be surprisingly effective in the context of methods of the invention is a regimen wherein the subject is provided with restricted amounts of a high-fat (HF) diet as sole food source (nutrition source). Appropriate drug treatments include treatment with agents such as the antihyperlipoproteinemic Acipimox™ (Olbetam™), i.e. 5-methylpyrazinecarboxylic acid 4-oxide, and related compounds (see US 4,002,750). Other classes of antihyperlipoproteinemic or lipid-reducing agents, including statins, such as Fluvastatin™, Lovastatin™, Pravastatin™ or Simvastatin™, and fibrates, such as Bezafibrat™, Clofibrat™ or Gemfibrozil™ may similarly be of value in this respect.

Another, related aspect of the present invention relates to a method for reducing blood lipid levels in an animal or human subject substantially without induction of hyperinsulinemia in the subject, the method comprising administering a growth hormone (GH) to the subject whilst providing the subject with restricted amounts of a high-fat (HF) diet as sole food source.

A still further aspect of the invention provides another method for reducing blood lipid levels in an animal or human subject substantially without induction of hyperinsulinemia in the subject, the method comprising administering a growth hormone (GH) to the subject whilst inhibiting lipolysis in the subject. In this connection, inhibition of lipolysis may be exemplified by inhibition of the lipase known as Hormone-Sensitive Lipase (HSL), although inhibition of other families of lipases may also be of relevance in the context of the method in question according to the invention. Substances capable of inhibiting the lipolytic effect of HSL are known (see, e.g., WO 01/17981 and WO 01/66531) and are anticipated as being applicable in this connection.

Yet another aspect of the invention relates to a method for reducing blood lipid levels in an animal or human subject substantially without induction of hyperinsulinemia in the subject, the method comprising administering a growth hormone (GH) to the subject whilst stimulating lipid clearance from the circulation. In this connection, stimulation of clearance of lipid from the circulation may, for example, be achieved by administration of a substance which acts to stimulate or activate a lipase such as Lipoprotein Lipase (LPL). Substances of this type are known, for example, from WO 01/27088.

With regard to what constitutes "restricted" amounts of a HF diet in the context of the invention, it is generally preferable that the energy content (caloric content) of the amount of HF diet with which the subject is provided does not exceed [i.e. is below or is equal to (or at least approximately equal to)] the theoretical maintenance level for the subject in question. In the case of human subjects, there is an extensive body of published data which enables the establishment of the theoretical maintenance level for an individual on the basis of parameters such as age, gender, weight, height, ethnicity and level of physical activity. Published sources of such data include: Ritz, P., *Factors affecting energy and macronutrient requirements in elderly people*, Public Health Nutrition 4 (2001) pp. 561-569; and Lin et al., *Estimation of energy requirements in a controlled feeding trial*, Am. J. Clin. Nutr. 77 (2003) pp. 639-645.

With regard to animal species, particularly "farm" animals (animals of importance in relation to production of meat products, dairy products, eggs and the like, such as cattle, pigs, goats and poultry), and other domestic animals, such as horses, data are available from sources such as the UK Agricultural Research Council (ARC), the Commonwealth Agricultural Bureau, and the US National

Research Council (NRC; e.g. data from 1988 and 1998, published by National Academy Press, Washington DC).

As already indicated to some extent elsewhere herein, it is clear that particularly important and valuable aspects of the present invention relate to the treatment of humans, in particular obese humans. In this connection, the growth hormone to be employed will likewise clearly preferably be human growth hormone (hGH).

In the light of the above methods of the invention, still further aspects of the present invention include the following:

pharmaceutical compositions comprising, as active ingredients, a growth hormone and an agent selected from: agents capable of reducing blood lipid levels; lipolysis-inhibiting agents (e.g. HSL inhibitors); and lipase-activating agents (e.g. LPL activators or potentiators);

medical kits suitable for use in methods according to the invention and comprising a growth hormone preparation and one or more measures which cause a reduction in blood lipid levels [such as a medical kit comprising a growth hormone preparation and a high-fat diet, a medical kit comprising a growth hormone preparation and a drug which causes a reduction in blood lipid levels, a medical kit comprising a growth hormone preparation and a lipolysis-inhibiting agent (such as an HSL inhibitor), or a medical kit comprising a growth hormone preparation and a lipolysis-activating agent (such as a LPL activator or potentiator).

The above-described aspects of the invention all relate to methods, compositions or medical kits involving a growth hormone *per se* (as defined herein; see below). An important further aspect of the invention relates, however, to the use of a substance which acts as a growth hormone secretagogue (GHS; also known, *inter alia*, as a growth hormone releasing substance) [i.e. a substance which when administered to a subject by an appropriate route is capable of stimulating the release of growth hormone from the pituitary of the subject] as an alternative to a GH *per se* in the various aspects of the invention (i.e. as an active ingredient in methods, pharmaceutical compositions, medical kits etc. as described above). Suitable substances of this type are, for example, described in WO 95/17423. Naturally occurring substances of this type, which are also of relevance in the context of the invention, include so-called growth hormone releasing peptides (GHRP's).

PHARMACEUTICAL ADMINISTRATION

The regimen for treatment of a given subject/patient with growth hormone and, where appropriate, with another drug, in the manner described herein, may be determined by one skilled in the art. The daily dose to be administered can be determined by a physician and will depend on the particular substance employed, on the route of administration and on the age and the condition of the subject or patient. A convenient daily dosage of GH is typically in the range of from about 0.001mg/kg body weight to about 2.0 mg/kg body weight, often from about 0.01 mg/kg body weight to about 1.0 mg/kg body weight. The therapeutic dose of the substance will depend upon the frequency and mode of administration, the sex, age, weight and general condition of the subject treated, the nature and severity of the condition treated and any concomitant diseases to be treated and other factors evident to those skilled in the art.

GH may be administered in a single dose or in repeated doses during the day. Administration in the manner described herein should continue until the treated individual is no longer in need of such treatment, for example, until an initially obese individual is no longer obese.

The route of GH administration may be any route that effectively transports the active compound to the appropriate or desired site of action, such as by infusion (continuous or pulsatile), injection, pulmonary inhalation, or by oral or nasal administration. Presently preferred routes include parenteral routes (e.g. via intramuscular, intraperitoneal, intravenous or subcutaneous injection, or by implant). The growth hormone can be formulated in dosage forms appropriate for each route of administration. The compositions or dosage forms may appear in conventional forms, e.g. aerosols, solutions, or suspensions.

A GH composition may be in a form suited for systemic injection or infusion, and may, as such, be formulated with a suitable liquid vehicle such as sterile water or an isotonic saline or glucose solution. The compositions may be sterilized by conventional sterilization techniques which are well known in the art. The resulting aqueous solutions may be packaged for use as such, or they may be filtered under aseptic conditions and lyophilized, the lyophilized preparation being combined with the appropriate sterile aqueous vehicle prior to administration. The composition may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as buffering agents, tonicity-adjusting agents and the like. Non-limiting examples include sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, and the like. Examples of liquid carriers are syrup, peanut oil, olive oil, phospholipids, fatty acids, fatty acid amines, polyoxyethylene and water.

It may be advantageous to provide GH in the form of a sustained release formulation. As such, the composition may be formulated as microcapsules or microparticles containing the growth hormone encapsulated in, or dispersed in, a suitable pharmaceutically acceptable biodegradable polymer, such as polylactic acid, polyglycolic acid or a lactic acid/glycolic acid copolymer.

For nasal administration, the GH preparation may contain growth hormone dissolved or suspended in a liquid carrier, in particular an aqueous carrier, for aerosol application. The carrier may contain additives such as solubilizing agents (e.g. propylene glycol), surfactants, absorption-enhancers such as lecithin (phosphatidylcholine) or cyclodextrin, or preservatives such as parabenes.

Growth hormone may be formulated by any of the established methods of formulating pharmaceutical compositions, e.g. as described in Remington: The Science and Practice of Pharmacy (1995).

DEFINITIONS

High-fat (HF) diet: Whilst it is difficult to arrive at a single definition of the term "high-fat" as used herein which is meaningful in relation not only to humans, but also to relevant animal species, a suitable definition for the purposes of the present invention as applied to humans is that given by M.R. Freedman et al. in a review article in Obesity Research 9, Suppl. 1 (March 2001) pp. 1S-40S. With reference to humans, the following table provides appropriate definitions not only of high-fat (HF) diets in the context of the present invention, but also of moderate-fat (MF), low-fat (LF) and very-low-fat (VLF) diets:

Table. Caloric composition of diets*

Diet type	Fat (% kcals)	Carbohydrate (% kcals)	Protein (% kcals)
High-fat (HF)	55-65	<20%	25-30
Moderate-fat (MF)	20-30	55-60	15-20
Low-fat (LF)	11-19	>65	10-20

*M.R. Freedman, J. King and E. Kennedy, *Popular diets: a scientific review*, Obesity Research 9, Suppl. 1 (March 2001) pp. 1S-40S

Obesity: the terms "obesity" and "obese" when employed in the context of the present invention imply an excess of adipose tissue. In this context obesity is best viewed as any degree of excess adiposity that imparts a health risk. The distinction between normal and obese individuals can only be approximated, but the health risk imparted by obesity is probably a continuum with increasing adiposity. However, in the context of the present invention, human individuals with a body mass index (BMI = body weight in kilograms divided by the square of the height in meters) above 25 are to be regarded as obese.

Growth hormone (GH): Growth hormone is a hormone that stimulates growth of all tissues capable of growing. Growth hormone is released from the pituitary. The release is under tight control of a number of hormones and neurotransmitters, either directly or indirectly. Growth hormone release can be stimulated by growth hormone releasing hormone (GHRH) and inhibited by somatostatin. In both cases, the hormones are released from the hypothalamus, but their action is mediated primarily via specific receptors located in the pituitary. In the present context "growth hormone" may be growth hormone from any origin, e.g. avian, bovine, equine, human, ovine, porcine, salmon, trout or tuna growth hormone. Human growth hormone (hGH) will normally be preferred for the treatment of humans. The growth hormone used in accordance with the invention may be native growth hormone isolated from a natural source, e.g. by extracting pituitary glands in a conventional manner, or a growth hormone produced by recombinant techniques, e.g. as described in E.B. Jensen and S. Carlsen in Biotech and Bioeng. 36 1990) pp. 1-11. The term growth hormone (GH) in the context of the invention also encompasses: truncated forms of GH, i.e. truncated forms of a growth hormone wherein one or more amino acid residues has/have been deleted; GH analogues, wherein one or more amino acid residues in the native molecule has/have been substituted with another amino acid residue, preferably a residue of a naturally occurring amino acid, as long as the substitution does not lead to any adverse effect such as antigenicity or reduced activity; and GH derivatives, e.g. deamidated or sulfoxidated forms of the growth hormone, or forms having an N- or C-terminal extension such as Met-hGH, Met-Glu-Ala-Glu-hGH or Ala-Glu-hGH. The preferred growth hormone for treatment of humans is normally hGH (i.e. natural human growth hormone, or recombinantly produced human growth hormone which is identical to the natural hormone), although methionylated human growth hormone may often be employed.

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CLAIMS

1. A method for substantially preventing hyperinsulinemia in an animal or human subject undergoing treatment with growth hormone (GH), the method comprising subjecting said subject, during the growth hormone treatment period, to one or measures which cause a reduction in blood lipid levels.

2. A method for achieving breakdown of adipose tissue in an animal or human subject substantially without induction of hyperinsulinemia in said subject, the method comprising administering a growth hormone (GH) to said subject whilst subjecting said subject to one or more measures which cause a reduction in blood lipid levels.

3. A method for reducing blood lipid levels in an animal or human subject substantially without induction of hyperinsulinemia in said subject, the method comprising administering a growth hormone (GH) to said subject whilst providing said subject with restricted amounts of a high-fat (HF) diet as sole food source.

4. A method for reducing blood lipid levels in an animal or human subject substantially without induction of hyperinsulinemia in said subject, the method comprising administering a growth hormone (GH) to said subject whilst inhibiting lipolysis in said subject.

5. A method according to claim 1 or 2, wherein said measures are selected from diet regimens and drug treatments.

6. A method according to claim 5, wherein said diet regimen entails providing said subject with restricted amounts of a high-fat (HF) diet as sole food source.

7. A method according to claim 3 or 6, wherein the energy content of said restricted high-fat diet does not exceed the theoretical maintenance level for said subject.

8. A method according to any one of claims 1-7, wherein said subject is a human.

9. A method according to claim 8, wherein said subject is an obese human.

10. A method according to claim 8 or 9, wherein said growth hormone (GH) is human growth hormone (hGH).

11. A pharmaceutical composition comprising, as active ingredients, a growth hormone and an agent capable of reducing blood lipid levels.

12. A pharmaceutical composition comprising, as active ingredients, a growth hormone and a lipolysis-inhibiting agent.

13. A pharmaceutical composition according to claim 12, wherein said agent is an HSL inhibitor.

14. A medical kit suitable for use in a method according to claim 1 or 2 and comprising a growth hormone preparation and one or more measures which cause a reduction in blood lipid levels.

15. A medical kit suitable for use in a method according to claim claim 3, 5 or 6, and comprising a growth hormone preparation and a high-fat diet.

16. A medical kit according to claim 14, comprising a growth hormone preparation and a drug which causes a reduction in blood lipid levels.

17. A medical kit suitable for use in a method according to claim 4 and comprising a growth hormone preparation and a lipolysis-inhibiting agent.

18. A medical kit according to claim 17, wherein said lipolysis-inhibiting agent is an HSL inhibitor.

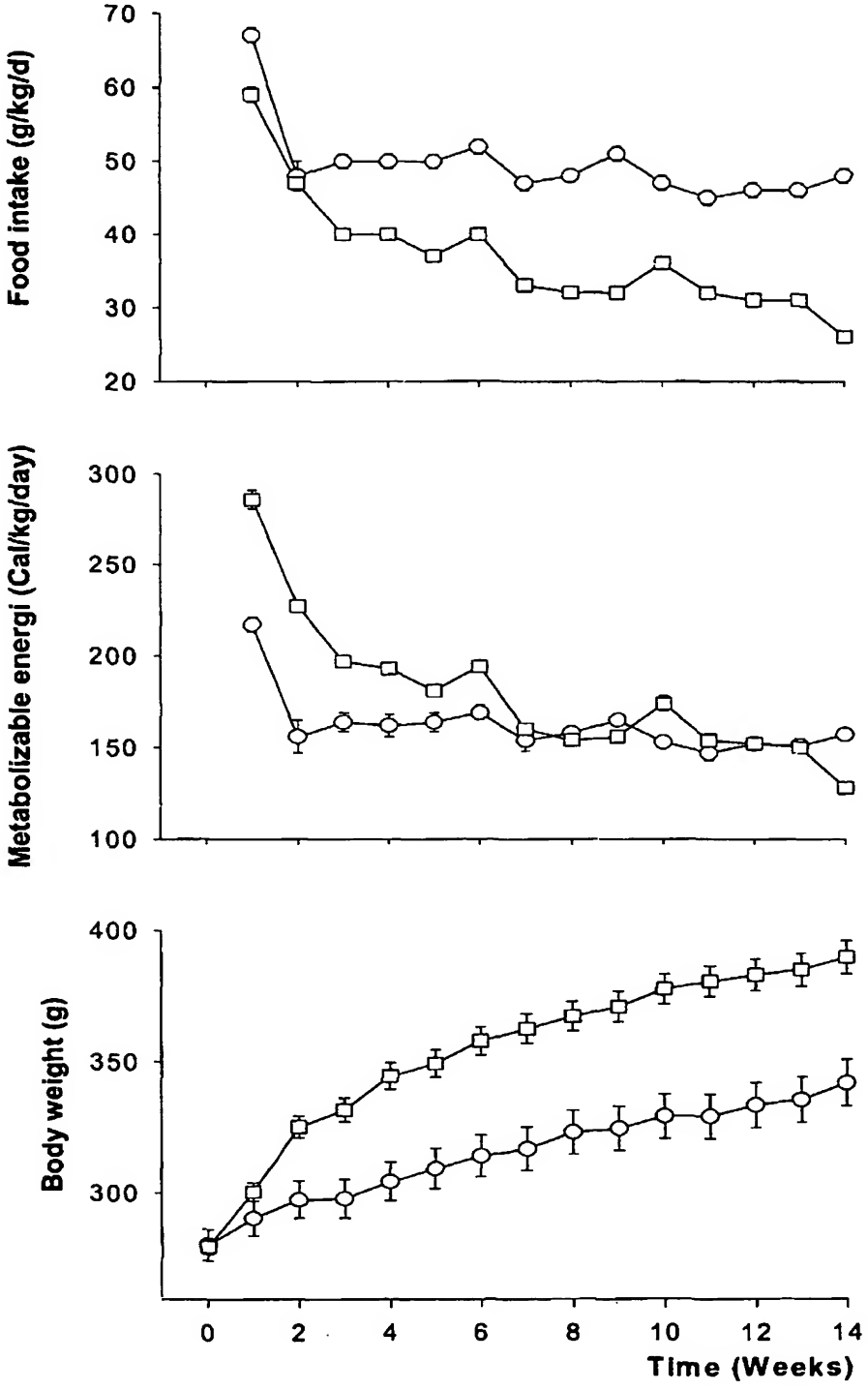

Fig. 1

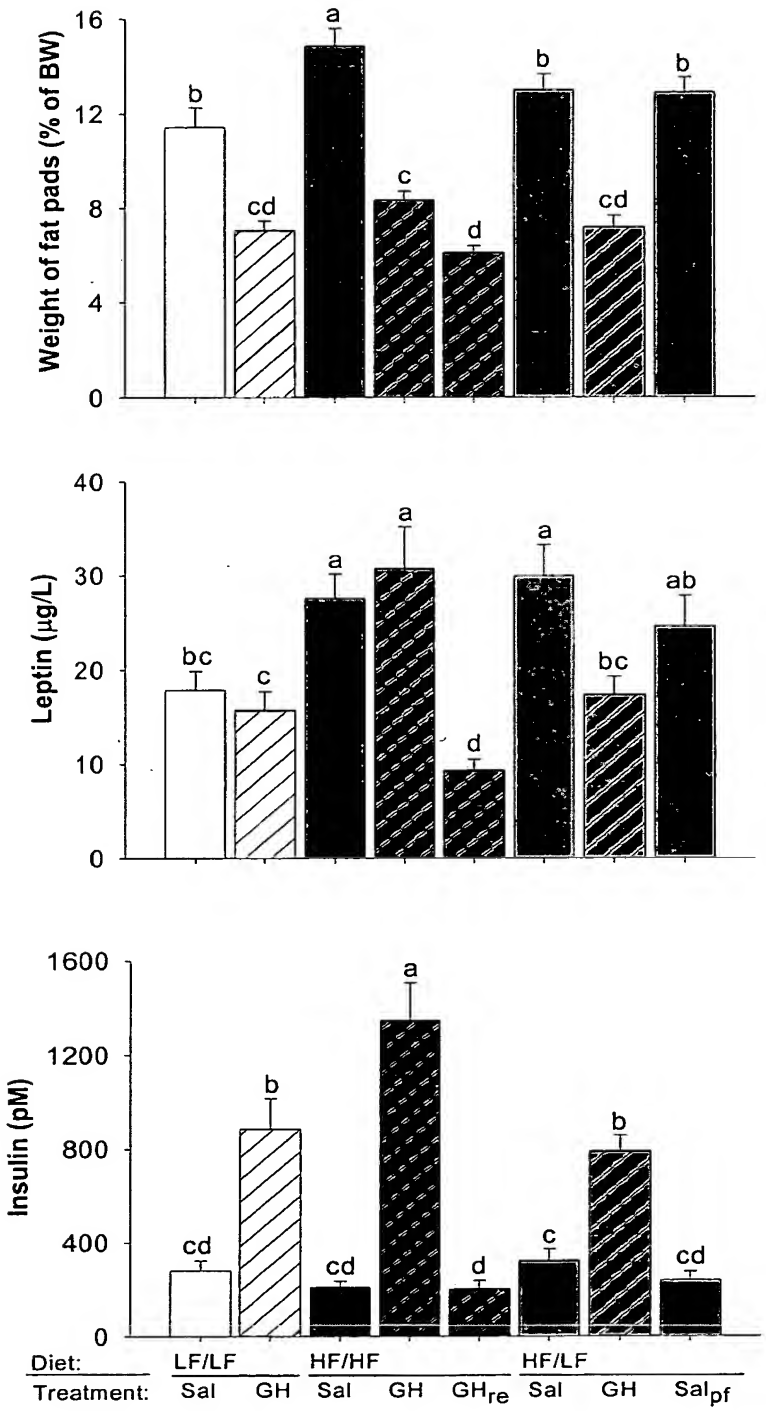

Fig. 2

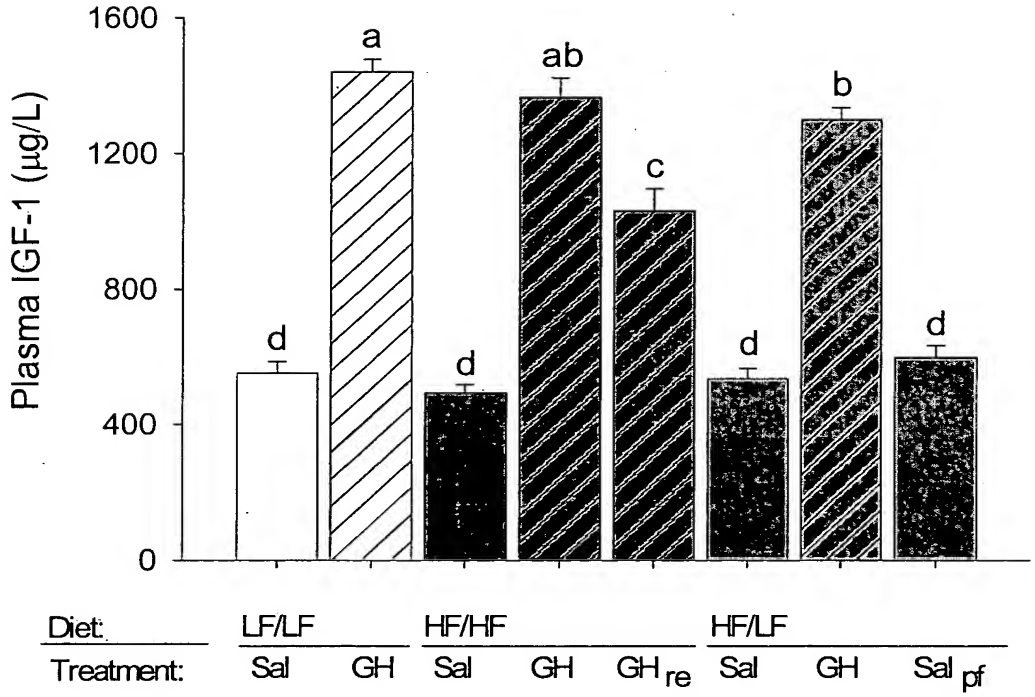

Fig. 3

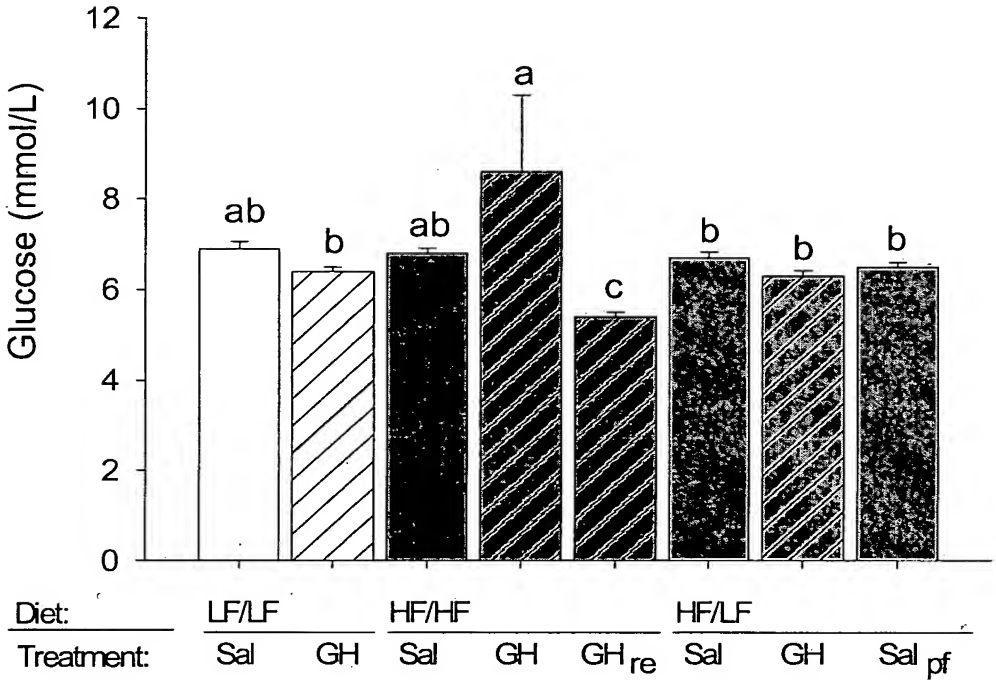

Fig. 4